The Canadian Society for Bioengineering
The Canadian society for engineering in agricultural, food, environmental, and biological systems.

La Société Canadienne de Génie Agroalimentaire et de Bioingénierie
La société canadienne de génie agroalimentaire, de la bioingénierie et de l'environnement

CSBE | SCGAB

Paper No. CSBE13-123

Biochemical Methane Potential for Wheat-based Fuel Ethanol and Beef Feedlot Integration

Holly Annand, M.Sc., P.Eng.

Associated Engineering, Calgary, AB, Holly.annand@usask.ca

Terrance Fonstad, Ph.D., P.Eng.

University of Saskatchewan, Civil & Geological Engineering, 57 Campus Drive, Saskatoon, SK S7N5A9, terry.fonstad@usask.ca

Dyan Pratt, M.Sc., P.Eng.

University of Saskatchewan, Civil & Geological Engineering, 57 Campus Drive, Saskatoon, SK S7N5A9, dyan.pratt@usask.ca

Tim Dumonceaux, Ph.D.

Agriculture & Agri-food Canada, 107 Science Pl, Saskatoon, SK S7N0X2, tim.dumonceaux@agr.gc.ca

Written for presentation at the
CSBE/SCGAB 2013 Annual Conference
University of Saskatchewan, Saskatoon, Saskatchewan
7-10 July 2013

ABSTRACT Biochemical methane potential (BMP) assays were conducted on byproducts from dry-grind wheat-based ethanol plants receiving two ratios of feedlot manure. Whole stillage, thin stillage and wet cake were tested alone and with 1:1 and 2:1 ratios (VS basis) of byproduct to feedlot manure. The resulting methane yield for ethanol byproducts with 1:1 VS ratio of manure were 389 ± 15, 446 ± 12 and 344 ± 12 ml CH_4/g VS added for whole stillage, thin stillage and wet cake, respectively. When ethanol byproducts were amended with feedlot manure at 2:1 VS ratio (byproduct:manure), the methane yields were 399 ± 18, 523 ± 13 and 367 ± 12 ml CH_4/g VS added for whole stillage, thin stillage and wet cake, respectively. Methane yield and production rate, total and volatile solids and pH were recorded for all experiments. With the exception of thin stillage, methane yields of ethanol byproducts reached expected values for manure amended versions based on the ratio of byproduct to manure. However, manure amended thin stillage produced 125% and 119 % of the expected methane yield for the 1:1 and 2:1 ratio experiments, respectively.

Keywords: anaerobic digestion, wheat ethanol byproducts, DDGS, manure, thin stillage, wet cake

INTRODUCTION As of September 1, 2010 the Canadian government adopted a renewable fuels standard that requires a 5% blend of ethanol into all sales of gasoline (CRFA, 2010). In Canada, fuel ethanol production is dominated by the fermentation and distillation of starchy grains like corn and wheat. The byproducts of ethanol production are processed and dried at high temperatures to create a high protein animal feed, dried distiller's grains with solubles (DDGS). For every liter of ethanol produced, between 8 and 15 liters of byproduct effluent requires processing (Saha et al., 2005). Energy consumed to produce DDGS decreases the net energy balance ratio of ethanol production and can negatively impact the carbon footprint of ethanol facilities.

As renewable fuel standards increase, the risk of DDGS market saturation increases. Increased fuel ethanol production also increases risks of pollution from CO_2 and organic loading in wastewaters. Anaerobic digestion of ethanol byproducts could potentially reduce the environmental impact of wastewaters leaving ethanol plants and improve the net energy balance ratio of the process if methane gas is combusted and returns heat and electricity to the process. The methane generating potential of wheat-based ethanol byproducts has not been widely published, so designing anaerobic digesters for ethanol plants is difficult. Information is more publicly available for corn-based ethanol byproducts, but it is not clear if the two types of byproducts have similar methane potentials.

Co-locating an ethanol plant and an anaerobic digester at a beef feedlot could provide synergistic economic and environmental advantages. Co-digestion of feedlot manure with ethanol byproducts is possible and ethanol byproducts can either be fed to the digester or to the cattle in the feedlot. Linking all three components (ethanol plant, beef feedlot and anaerobic digester) creates what is known as a biorefinery where the byproducts of one entity become the inputs for the next and the overall system operates in concert as shown below in Figure 1.

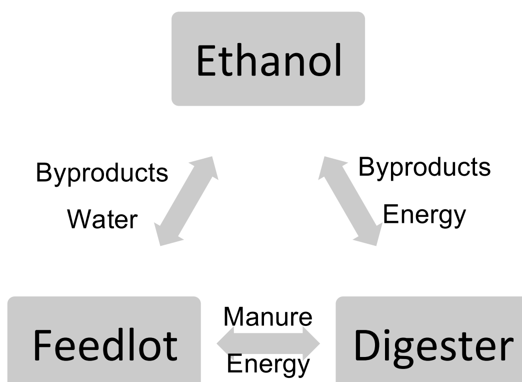

Figure 1. Flow of materials through proposed biorefinery

Wheat-based ethanol production

Figure 2 is a general schematic of the ethanol production process. Downstream processing starts after distillation and can be a major limitation to ethanol production since it consumes approximately 47% of the plants total energy needs (Eskicioglu et al., 2011). Bottlenecks in downstream processing can also disrupt system balances and hold up ethanol production on the front end. There are typically six byproduct streams that are generated during downstream processing. These streams are whole stillage, thin stillage, wet cake, syrup, wet distiller's grains with solubles (WDGS) and dried distillers grains with solubes (DDGS).

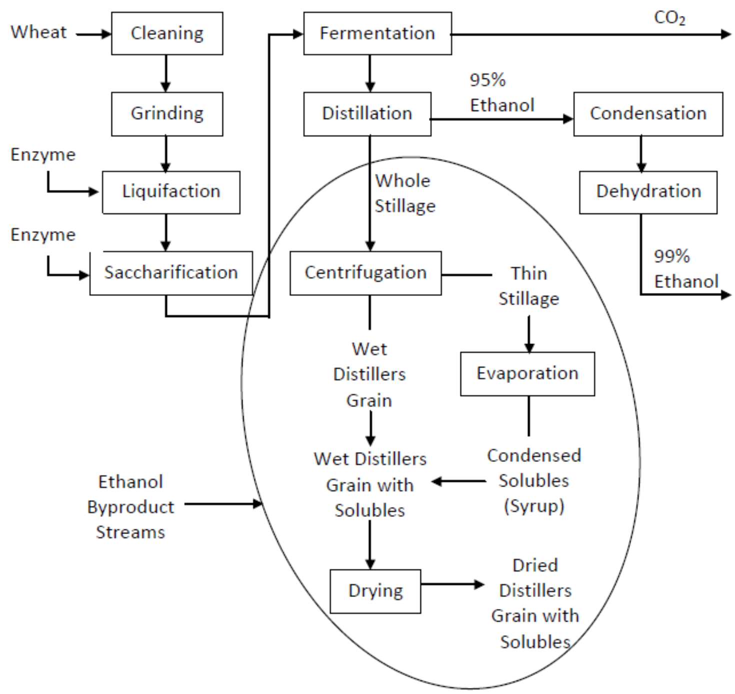

Figure 2. Ethanol production process highlighting byproduct streams

Anaerobic digestion of ethanol byproducts

Many studies have been published on anaerobic digestion of corn ethanol byproducts. Research has primarily been focused on thin stillage because it requires the most processing energy and it presents the greatest risks to wastewater leaving ethanol plants. In the 1980's, two mesophilic studies on corn thin stillage reported methane yields of 250 –370 ml CH_4/g total chemical oxygen demand (TCOD) removed (Stover et al., 1984) and 330 ml CH_4/g TCOD removed (Lanting and Gross, 1985). Stover et al. (1984) used suspended growth and fixed film reactors and suggested that this methane production volume could supply 60% of the daily energy consumed by ethanol plants. Lanting and Gross (1985) used up-flow anaerobic sludge blanket (USAB) reactors.

More recent, research has focused on digesting corn thin stillage at thermophilic temperatures. Thermophilic digestion is sometimes considered a disadvantage compared to mesophilic digestion because it requires more energy to reach higher heating temperatures. In ethanol plants, however, whole stillage exits the distillation column at above 55°C, so heating demands are decreased and the metabolic rates achieved by thermophilic digestion provide improved efficiency and economics (Agler et al., 2008; Schaefer and Sung, 2008).

Using completely mixed thermophilic reactors at 30-, 20-, and 15-day hydraulic retention times (HRT) and thin stillage concentrated at 100 g/L TCOD and 60 g/L VS, Shafer and Sung (2008) observed methane yields ranging between 600 – 700 ml CH_4/g VS removed. They suggested that natural gas consumption at ethanol plants could be reduced by 43 to 59% with this level of methane production. Another thermophilic study on thin corn stillage used high rate sequencing batch reactors to realize a methane yield of 254 ml CH_4/g TCOD fed on a 10 day HRT (Agler et al., 2008). Anger et al. (2008) suggested that this amount of methane production would reduce natural gas consumption in conventional dry grind ethanol plants by 51% and translate to an improved net energy balance ratio of ethanol from 1.26 to 1.70.

In 2011, Eskicioglu et al. published a study of batch and continuous-flow experiments where whole corn stillage was digested under thermophilic and mesophilic conditions. BMP assays produced methane volumes of 401 ± 17, 406 ± 14, 441 ± 2 and 458 ± 0 ml CH_4/g VS added under mesophilic and 693 ± 17, 560 ± 24, 529 ± 37 and 429 ± 8 ml CH_4/g VS added under thermophilic conditions for whole corn stillage at concentrations of 6348, 12,696, 25,393, and 50,786 mg TCOD/L, respectively (Eskicioglu et al., 2011). Little success was realized during continuous flow experiments with full strength whole corn stillage (254 g TCOD/L) at organic loading rates of 4.25, 6.30 and 9.05 g TCOD/L day. At thermophilic temperatures, the digesters were unstable and at mesophilic temperatures, only a 60 day retention time was stable.

Anaerobic digestion of manure

Manure is a widely used feedstock for anaerobic digestion because it decreases the volume of green house gas emissions released during normal manure storage (Moller et al., 2004). Manure itself is a good substrate for co-digestion with other organic material because it can adjust the carbon-to-nitrogen (C:N) ratio of feedstock (25-30:1 optimal), it can provide buffering capacity (alkalinity) and it can supply essential nutrients that improve methane yields (Labatut and Scott, 2008; Ward et al., 2008). The biogas potential of manure is highly variable and it depends on the type of animal, the animal's feed, climate conditions and the type of bedding used, not to mention the storage conditions of manure before anaerobic digestion occurs (Moller et al., 2004). A typical specific methane yield of beef cattle manure is 328 ml/g VS added (Hashimoto et al., 1981).

Objective

The objective of this study was to determine the effect of feedlot manure on the biochemical methane potential of wheat ethanol byproducts under thermophilic conditions.

METHODS AND MATERIALS

Biochemical methane potential (BMP) assay

BMP assays similar to Owen et al. (1979) and Angelidake et al., (2009) were performed under thermophilic (55 ± 2°C) conditions in this study. Using predetermined total solids (TS) and volatile solids (VS) content of the various substrates, mixtures of 5% TS and 1:1 VS ratio (substrate:inoculum by mass) were prepared. When feedlot manure amendments were added, the substrate:inoculum VS ratio remained 1:1 and the substrate itself was composed of either 1:1 or 2:1 VS ratios of ethanol byproduct to manure. 300 ml of each mixture was loaded into 1 litre glass assay bottles which were then sealed with a rubber septum and screw cap as shown in Figure 3. Samples were taken to measure the actual TS, VS and pH of each prepared mixture. The resulting headspace of the sealed bottles was flushed with pure nitrogen gas for 5 minutes at room temperature. Finally, the assay bottles were loaded into a thermophilic (55 ± 2°C) incubator.

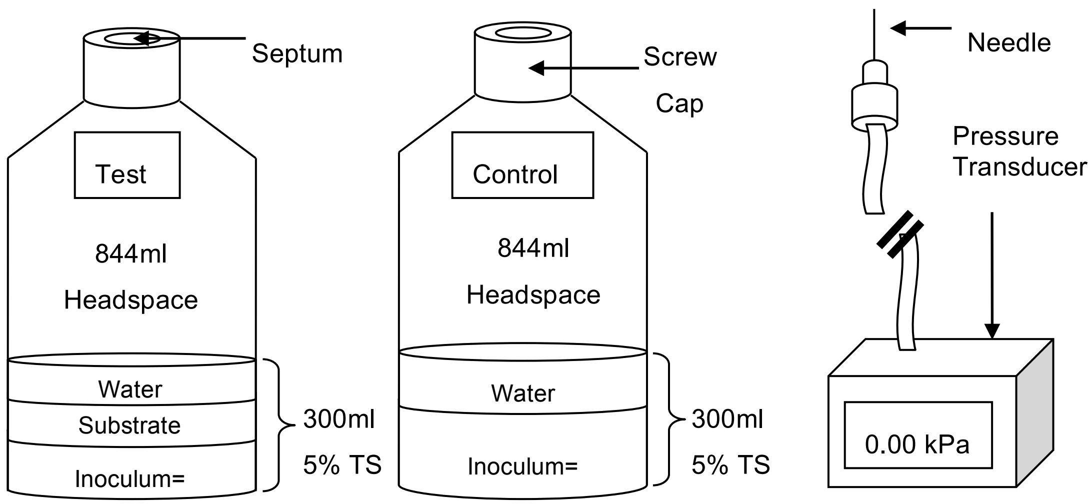

Figure 3. Set up for BMP assays

Biogas production was monitored for every bottle throughout the duration of each experiment. Bottles had to be removed from the incubator, but measurements of biogas volume where done immediately with a needle pressure transducer and calculations were made to convert pressure readings into biogas volumes at standard temperature and pressure. Each time biogas volumes were measured, a 20 ml sample of gas was taken using a needle syringe. The sample was then transferred to a 5 ml evacuated vial for analysis by gas chromatography (GC). After the biogas samples were taken, the bottles were vented down to a known pressure, swirled gently and placed back into the incubator.

Analytical methods

Biogas samples were analyzed using gas chromatography (GC). The relative percentages of methane, carbon dioxide, hydrogen, nitrogen and oxygen were determined using a Varion model 450-GC with front and middle TCD detectors. Injector, oven and detector temperatures were 100°C, 50°C and 150°C, respectively. The front column was a Hayesep Q 80/100 CP81069 (1 m x 3.175 mm) using argon make up gas flowing at 20 ml/min. The middle column was a Molsieve 5A 80/100 CP81025 (1 m x 3.175 mm) using helium make up gas flowing at 20 ml/min. The standard gas used for calibrating the GC was composed of H_2(0.5%), CH_4(40%), N_2(1%), O_2(5%), CO_2(bal%).

When the daily biogas production volume dropped below one percent (1%) of the total biogas produced up to that date, the experiment was ended. The bottles were opened and the digestate analyzed for TS and VS. The pH of each bottle was also measured.

TS and VS were determined by standard methods (APHA, 1995) with provisions made to avoid losing volatile solids during TS determination. As per Angelidaki et al. (2009) recommendations, TS determination was performed at 70°C, until constant weight (48 hours).

Angelidaki et al. (2009) suggest that specific methane production can be reported as volume of CH_4 per gram VS, or CH_4 per gram COD or CH_4 per gram of sample. In this study, COD was not measured and results are reported as CH_4 per gram VS added to the bottles. Methane production rate, *k*, was determined by the slope of the linear curve obtained when experimental data was plotted according to Equation 1 (Angelidaki et al., 2009). The coefficient of determination, R^2, was calculated to show how well the data followed first order rate kinetics.

$$ln\frac{(B_o - B)}{B_o} = -kt \qquad \text{Equation 1}$$

Substrate inoculum characterization

Ethanol byproducts were sampled from a dry-grind wheat-based ethanol plant in Saskatchewan. Samples were collected and then stored at 4°C until needed. TS and VS of each sample were measured to determine quantities needed in each experiment. Ethanol byproducts tested in the 1:1 experiment had been stored at 4°C for more than 30 days, while the byproducts for the 2:1 experiment were stored for less than one week.

Manure samples were collected from an Alberta beef feedlot for the 1:1 experiment and from a Saskatchewan beef feedlot for the 2:1 experiment. The Alberta feedlot manure sample was collected in the late spring, transported in less than two hours and stored at 4°C until used. The Saskatchewan sample was collected in the early fall and was in transport for approximately twelve hours before being stored at 4°C until needed.

The same inoculum was used for both experiments in the study. The inoculum was obtained from an anaerobic digester while it was operating primarily on feedlot manure. The TS and VS of the inoculum were determined before it was frozen at -20°C until needed. The inoculum was thawed and incubated 5 and 7 days prior to the beginning of each round of experiments. Table 1 lists the TS, VS and VS/TS ratio for ethanol byproducts, manures and inoculum used in each experiment.

Table 1: Characterization of wheat ethanol byproducts, manure and inoculum (n=3)

Experiment	Substrate	% TS	% VS	% VS/TS
1:1	Whole Stillage	17.68 ± 0.75	16.26 ± 0.73	91.94 ± 5.66
	Thin Stillage	15.79 ± 0.03	14.07 ± 0.46	89.08 ± 2.90
	Wet Cake	32.45 ± 0.44	31.37 ± 0.42	96.69 ± 1.83
	Manure	32.59 ± 4.46	23.63 ± 3.48	72.51 ± 14.57
2:1	Whole Stillage	19.13 ± 1.31	17.72 ± 1.42	92.62 ± 9.77
	Thin Stillage	13.33 ± 0.11	11.77 ± 0.41	88.29 ± 3.14
	Wet Cake	34.07 ± 0.16	33.01 ± 0.17	96.89 ± 0.67
	Manure	38.66 ± 3.16	17.53 ± 0.86	45.34 ± 4.33
	Inoculum[a]	9.42	6.43	68.25

[a] Limited volumes of inoculum were available, so TS and VS measurements made during another experiment were used and error data was not available.

Experimental set up

Two BMP experiments were performed to determine the ultimate methane yield and methane production rate that could be achieved from ethanol byproducts receiving two different ratios of feedlot manure. Based on the results from a previous experiment by Annand et al. (2011), whole stillage, thin stillage and wet cake received manure amendments of 1:1 and 2:1 VS ratios (byproduct:manure). Methane production of manure amended ethanol byproducts was compared to the results achieved by Annand et al. (2011) for un-amended byproducts.

RESULTS AND DISCUSSION Methane yields were corrected to account for endogenous metabolism of the inoculum which was determined by running control assays in each experiment. Methane production profiles are used to show the mean accumulated methane yield, B_o, across replicates in each experiment and error bars represent the standard deviation at each sampling interval. Data points from the methane production profiles were plotted according to Equation 4.1 to determine the methane production rate, *k*, and regression analysis was used to describe the fit of the data to first-order rate kinetics.

1:1 Experiment

Two graphs are presented to show how a 1:1 VS ratio manure amendment affected the methane potential of ethanol byproducts. Figure 4 shows methane production profiles for whole stillage, thin stillage and wet cake without manure amendment. Methane yields for these byproducts were 578 ± 14, 473 ± 59 and 493 ± 32 ml CH_4/g VS added, respectively. Figure 5 shows methane production profiles for whole stillage, thin stillage and wet cake with manure amendments as well as one for manure itself. Methane yields for these byproducts with 1:1 VS ratio manure and for manure alone were 389 ± 15, 446 ± 12, 344 ± 12 and 230 ± 16 ml CH_4/g VS added, respectively.

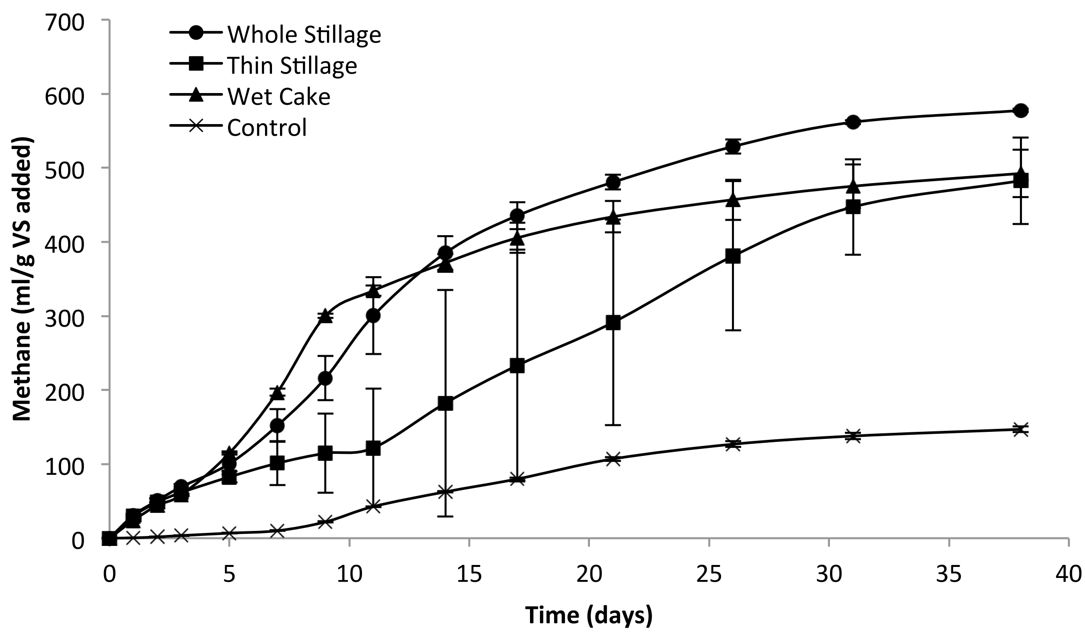

Figure 4: Un-amended methane production profiles

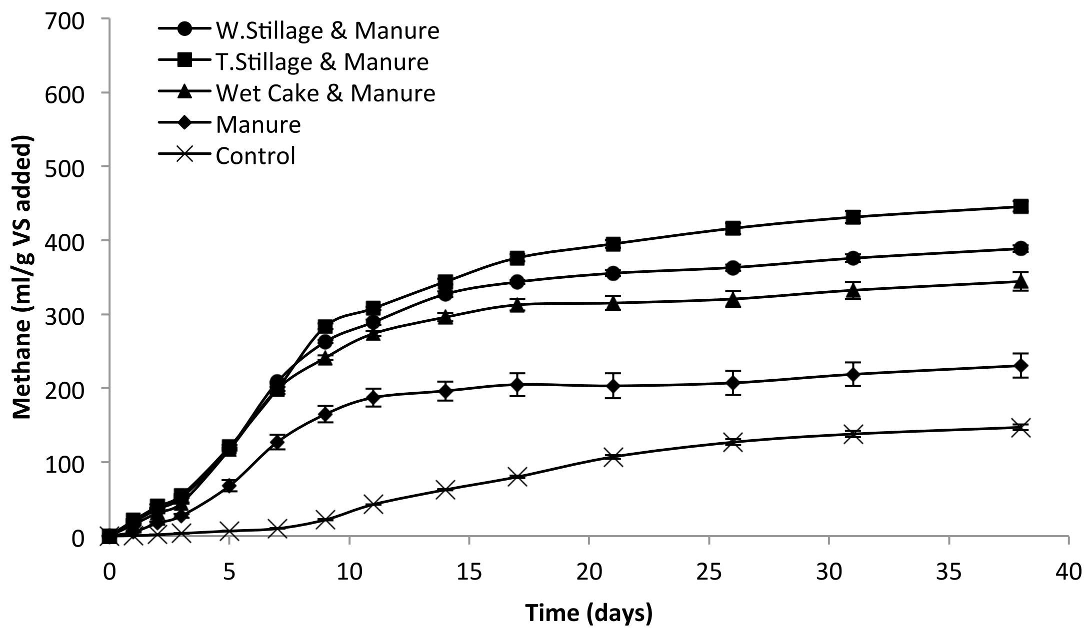

Figure 5: Manure amended methane production profiles (1:1 VS ratio)

The effect of manure amendment on ethanol byproduct methane potential can be seen by comparing Figures 4 and 5. As expected, methane yields are less in Figure 5 because the biogas potential of manure is less than that of ethanol byproducts. Manure amendment had a very obvious stabilizing effect on ethanol byproducts, especially thin stillage. The variability observed between bottles in Figure 4 was virtually eliminated in Figure 5 and thin stillage became the top methane yielding byproduct instead of whole stillage when manure was added. Table 2 provides the values for methane yield, B_o, methane production rate, k, and pH measurements determined in this 1:1 VS ratio experiment.

Table 2: 1:1 Experiment methane yield, methane production rate and pH

	Methane yield	Methane production rate		pH	
Substrate	B_o (ml/g VS added)	k (day^{-1})	R^2	Initial	Final
Whole Stillage	578 ± 14	0.094	0.927	7.76	7.81
Thin Stillage	483 ± 59	0.058	0.821	7.37	7.89
Wet Cake	493 ± 32	0.102	0.987	7.75	7.66
Whole Stillage & Manure	389 ± 15	0.113	0.980	7.97	7.59
Thin Stillage & Manure	446 ± 12	0.110	0.989	7.67	7.64
Wet Cake & Manure	344 ± 12	0.115	0.958	7.97	7.55
Manure	230 ± 16	0.104	0.915	8.19	7.45
Control	147 ± 4	NA		8.01	7.87

Figure 5 also shows much faster methane production rates than Figure 4; a fact that is reiterated by the methane production rate, *k*, values in Table 4.2. Whole stillage and thin stillage methane production did not fit first order kinetics very well (low R^2 values), but the manure amended versions of these byproducts did. Wet cake followed first order kinetics well, but the manure amended wet cake achieved an even faster methane production rate, 0.102 day^{-1} compared to 0.115 day^{-1}, respectively. The pH values of manure amended byproducts also started higher than their un-amended counterparts, which may have led to faster methane production rates caused by improved buffering capacity and micronutrient availability.

2:1 Experiment

Two graphs are presented to show how a 2:1 VS ratio of ethanol byproduct to manure affected the methane potential of ethanol byproducts. Figure 6 shows methane production profiles for whole stillage, thin stillage and wet cake without manure amendment. Methane yields for these byproducts were 533 ± 18, 592 ± 37 and 485 ± 19 ml CH_4/g VS added, respectively. Figure 7 shows methane production profiles for whole stillage, thin stillage and wet cake with manure amendments as well as one for manure itself. Methane yields for these byproducts receiving manure amendment and for manure alone were 399 ± 18, 523± 13, 367 ± 12 and 136 ± 12 ml CH_4/g VS added, respectively.

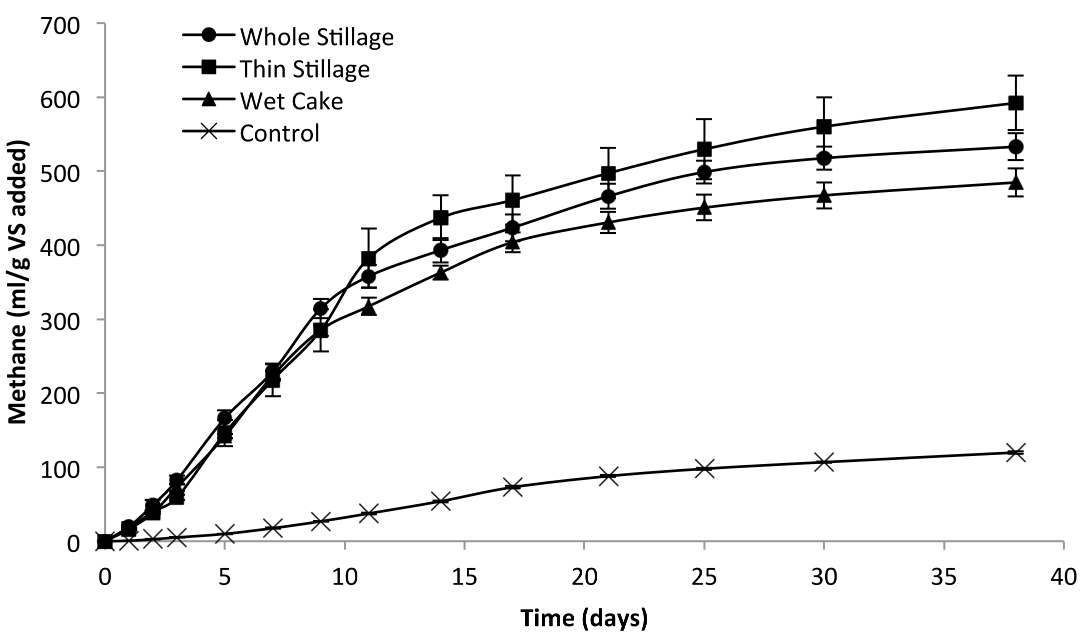

Figure 6: Un-amended methane production profiles

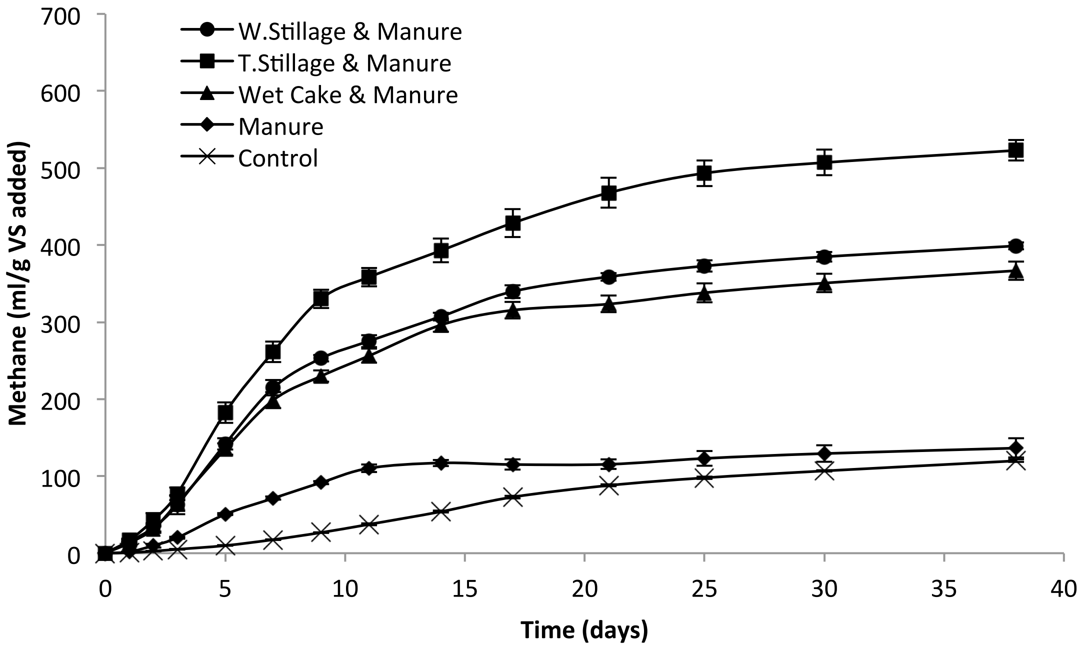

Figure 7: Manure amended methane production profiles (2:1 VS ratio)

The effect of manure amendment on ethanol byproduct methane potential can be seen by comparing Figures 6 and 7. It was again expected that methane yields would be less in Figure 7 because the biogas potential of manure is less than that of ethanol byproducts. In contrast to the 1:1 Experiment discussed above, methane production of un-amended ethanol byproducts was relatively stable in this experiment and thin stillage yielded the highest methane volumes in both the un-amended and manure amended trails. Table 3 provides the values for methane yield, B_o, production rate, k, and pH determined in the 2:1 Experiment.

Table 3: 2:1 Experiment methane yield, methane production rate and pH

	Methane yield	Methane production rate		pH	
Substrate	B_o (ml/g VS added)	k (day^{-1})	R^2	Initial	Final
Whole Stillage	533 ± 18	0.106	0.977	7.41	7.70
Thin Stillage	592 ± 37	0.090	0.983	7.31	7.78
Wet Cake	485 ± 19	0.105	0.990	7.58	7.57
Whole Stillage & Manure	399 ± 18	0.109	0.995	7.68	7.59
Thin Stillage & Manure	523 ± 13	0.110	0.988	7.71	7.66
Wet Cake & Manure	367 ± 12	0.105	0.990	7.81	7.44
Manure	136 ± 12	0.102	0.933	8.18	7.42
Control	120 ± 2	NA		8.03	7.84

Numbers in parentheses are R^2 values from regression analysis of k as determined from graphing experimental data.

Faster methane production rates can be seen by the earlier rise in methane production profiles in Figure 7, compared to Figure 6. Table 3 also shows that methane production rates, *k*, were faster for manure amended ethanol byproducts and that all the trials in this experiment followed first order reaction kinetics as demonstrated by high R^2 values, except for manure. Manure amendment caused thin stillage methane production rate to increase by 0.02 day^{-1}, which could shorten its required digester retention time by two days. The pH values of manure amended byproducts also started higher than their un-amended counterparts, which may have led to faster methane production rates caused by improved buffering capacity and micronutrient availability.

Summary and significance

Table 4 outlines the actual and expected methane yields of manure amended ethanol byproducts for both experiments. For the 1:1 experiment the expected methane yields are half of the ethanol byproduct yield plus half of the manure yield. For the 2:1 experiment the expected methane yields are two thirds of the ethanol byproduct yield plus one third of the manure yield. The manure amended byproduct yields are also expressed as a percent of the un-amended trials. This shows that offsetting ethanol byproduct use for manure still results in similar methane yields, especially for thin stillage.

Table 4: Effect of manure amendment on methane yield

		Methane yield, B_o (ml/g VS added)			
Experiment	Substrate	actual	expected	% of expected	% of un-amended
1:1	Whole Stillage & Manure	389 ± 15	404 ± 21	96%	67%
	Thin Stillage & Manure	446 ± 12	357 ± 35	**125%**	**92%**
	Wet Cake & Manure	344 ± 12	362 ± 24	95%	70%
2:1	Whole Stillage & Manure	399 ± 18	401 ± 23	100%	75%
	Thin Stillage & Manure	523 ± 13	440 ± 32	**119%**	**88%**
	Wet Cake & Manure	367 ± 12	369 ± 22	100%	76%

Amending thin stillage with feedlot manure produced 125% and 119% more methane than expected in the 1:1 and 2:1 experiments, respectively. The other ethanol byproducts responded as expected to manure amendments and produced methane at the ratios of their respective inputs. Higher fractions of manure in the 1:1 experiment produced less methane gas for whole stillage and wet cake, but the opposite occurred for thin stillage. Manure amended thin stillage produced 92% of the methane produced by thin stillage alone in the 1:1 experiment versus 88% in the 2:1 experiment. Theoretically, more of the higher producing ethanol byproduct would have been available for conversion to methane in the 2:1 experiment.

Possible explanations for this situation are that in the 1:1 experiment un-amended thin stillage may not have reached its full methane potential in the 38 day duration of the experiment. Thin stillage methane production was also slow start and highly variable in the 1:1 experiment, so manure may have supplied the necessary nutrients and microbial stability that allowed the amended version to perform so well.

CONCLUSION In a 1:1 VS ratio experiment whole stillage, thin stillage and wet cake responded to the addition of feedlot manure to achieve methane yields of 389 ± 15, 446 ± 12 and 344 ± 12 (ml CH_4/g VS added), respectively. In a 2:1 VS ratio experiment (byproduct:manure) whole stillage, thin stillage and wet cake responded to feedlot manure addition with methane yields of 399 ± 18, 523 ± 13 and 367 ± 12 (ml CH_4/g VS added), respectively. Feedlot manure stabilized the anaerobic digestion process and improved the methane production rates for all ethanol byproducts in this study.

A synergistic co-digestion relationship was exposed between feedlot manure and thin stillage. Equal parts of feedlot manure and thin stillage produced 92% of the methane produced by thin stillage alone. Similarly, 2 parts thin stillage were offset by 1 part feedlot manure and produced 88% of the methane produced by thin stillage alone. Adding feedlot manure to BMP assays of ethanol byproducts resulted in expected methane volumes from whole stillage and wet cake, but unexpectedly high volumes of methane from thin stillage.

REFERENCES

Agler, M., Garcia, M., Lee, E., Schilicher, M., & Angenent, L. (2008). Thermophilic anaerobic digestion to increase the net energy balance of corn grain ethanol. *Environmental Science & Technology , 42*, 6723-6729.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., et al. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science & Technology , 59.5*, 927-934.

Annand, H. 2011. Biochemical Methane Potential for Wheat-Based Fuel Ethanol and Beef Feedlot Integration. M.Sc. Thesis, University of Saskatchewan, Chemical & Biological Engineering.

Canadian Renewable Fuels Association (CRFA). (2010). *Ethanol - Key Issues. Fact Sheets/Responses to Key Issues for Canada's Ethanol Industry.* Retrieved March 28, 2011, from CRFA Resource Centre: http://www.greenfuels.org/uploads/documents/ethanol-fact-sheet.pdf

Eskicioglu, C., Kennedy, K. J., Marin, J., & Strehler, B. (2011). Anaerobic digestion of whole stillage from dry-gring corn ethanol plant under mesophilic and thermophilic conditions. *Bioresource Technology , 102*, 1079-1086.

Hashimoto, A. G., Varel, V. H., & Chen, Y. R. (1981). Ultimate methane yield from beef-cattle manure - effect of temperature, ration constituents, antibiotics and manure age. *Agricultural Wastes , 3*, 241-256.

Labatut, R. A., & Scott, N. R. (2008). Experimental and predicted methane yields from the anaerobic co-digestion of animal manure with complex organic substrates. *2008 ASABE Annual International Meeting.* Providence, Rhode Island: ASABE Paper No: 085087.

Lanting, J., & Gross, R. L. (1985). Anaerobic pretreatment of corn ethanol production wastewater. *40th Industrial Waste Conference* (pp. 905-914). Purdue University, West Lafayette, Indiana: Butterworth, Boston.

Moller, H. B., Sommer, S. G., & Ahring, B. K. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biomass & Bioenergy , 26*, 485-495.

Owen, W., Stuckey, D., Healy, J., Young, J. L., & McCarty, P. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research , 13*, 485-492.

Saha, N. K., Balakrishnan, M., & Batra, V. S. (2005). Improving Industial Water Use: Case Study for an Indian distillery. *Resource Conservation Recycling , 43*, 163-174.

Schaefer, S. H., & Sung, S. (2008). Retooling the ethanol industry: thermophilic anaerobic digestion of thin stillage for methane production and pollution prevention. *Water Environment Research , 80* (2), 101-108.

Stover, E. L., Gomathinayagam, G., & Gonzalez, R. (1984). Use of methane from anaerobic treatment of stillage for fuel alcohol production. *39th Industrial Wate Conference* (pp. 95-104). Purdue University, West Lafayette, Indiana: Butterworth, Boston.

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology , 99*, 7928-7940.